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Chemical and Physical Properties of Kiwifruit (*Actinidia deliciosa*) Starch*

Chemical and physical properties of kiwifruit (*Actinidia deliciosa* var. 'Hayward') starch were studied. Kiwifruit starch granules were compound, irregular or dome-shaped with diameters predominantly 4–5 μm or 7–9 μm. Kiwifruit starch exhibited B-type X-ray diffraction pattern, an apparent amylose content of 43.1% and absolute amylose content of 18.8%. Kiwifruit amylopectins, relative to other starches, had low weight-average molecular weight (7.4×10^7), and gyration radius (200 nm). Average amylopectin branch chain-length was long (DP 28.6). Onset and peak gelatinization temperatures were 68.9°C and 73.0°C, respectively, and gelatinization enthalpy was high (18.5 J/g). Amylose-lipid thermal transition was observed. Starch retrograded for 7 d at 4°C had a very high peak melting temperature (60.7°C). Peak (250 RVU), final (238 RVU) and setback (94 RVU) viscosity of 8% kiwifruit starch paste was high relative to other starches and pasting temperature (69.7°C) was marginally higher than onset gelatinization temperature. High paste viscosities and low pasting temperature could give kiwifruit starch some advantages over many cereal starches.

Keywords: Kiwifruit; *Actinidia*; Starch structure; Starch function; Amylose; Amylopectin; Kiwi fruit

1 Introduction

Starch is the most abundant carbohydrate of storage organs. Extensive research has been conducted on starch in cereals, legumes, root and tuber crops but little research has focused on the starches of fruit crops. Kiwifruit, like many fruit crops, accumulates starch during the early stages of fruit development but then degrades starch rapidly as fruit maturity approaches. Kiwifruit are always commercially harvested when sufficient starch has degraded for Brix value of fruit to reach about 6.2 [1], but starch is still predominant.

The starch characteristics of kiwifruit may provide useful information about texture of fresh fruit and in processing. Starch structure and functional properties have been previously shown to influence texture of winter squash [2] and potatoes [3]. Additionally, premature softening during storage is an expensive cost for the kiwifruit industry due to fruit loss and repackaging of firm fruit. Causes of kiwifruit premature softening are still unknown. Starch structure may play a role, as a high proportion of starch is degraded during premature softening of kiwifruit [4].

Kiwifruit starch characteristics have not been extensively researched but there has been one previous study investigating three kiwifruit varieties including Hayward variety [5]. The authors reported that kiwifruit starch from fruit at harvest has B-type polymorphism, a predominant granules diameter of 6–8 μm, and an amylose content of 14–21% based on amperometric iodine titration method of whole starch. Onset gelatinization temperature of kiwifruit starch was reported to be 62.4°C and enthalpy change of gelatinization was 4.3 cal/g (18.0 J/g).

In this study starch structural and functional characteristics of one kiwifruit variety were investigated to determine how similar kiwifruit starch properties are to that of other starches and if there are any unique characteristics that could be of industrial importance.

2 Materials and Methods

2.1 Plant material

Kiwifruit [*Actinidia deliciosa* (A. Chev.) C. F. Liang et A. R. Ferguson, var. deliciosa 'Hayward'] were harvested on October 29, 2003 at the Kearney Field Station, Kearney,

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* Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

CA. Three replicates, each consisting of 40 fruit collected randomly from vines, were harvested from both the northwest and southeast corners of one field, and fruit harvested from a single row of an adjacent field.

2.2 Methods

2.2.1 Starch isolation and quantification, and water content

Starch was isolated from kiwifruit using a method reported by *Kasemsuwan* et al. [6] with further modification by *Stevenson* [2]. Kiwifruit were stored for 2 d at 4°C after harvest, then fruit were sliced and blended in 0.3% (w/v) sodium metabisulfite using a Waring commercial blender (Waring Corp., New Hartford, CT, high mode used). Kiwifruit starch puree was then filtered through a screen of 106 μm mesh and the filtrate was centrifuged at 10,500 × *g* for 40 min to deposit starch. To remove protein, lipids and chlorophyll, the starch pellet was washed with 10% toluene in 0.1 M aqueous sodium chloride and left standing for at least 4 h. This step was repeated 6–10 times. The toluene/salt solution treated starch was then washed three times with deionized water, twice with ethanol and then recovered by filtration using Whatman no. 4 filter paper. The purified starch cake was dried in a convection oven at 35°C for 48 h. Water content of kiwifruit was determined by freeze-drying finely diced fruit. Total starch content of freeze-dried kiwifruit powders, measured in duplicate, was determined using the total starch assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland), based on AOAC method 996.11, AACC method 76.13 and ICC standard method No. 168, in which fruit powders are hydrolyzed with α-amylase and amyloglucosidase, and subsequent glucose content determined using glucose oxidase-peroxidase. An internal standard of corn starch was added to the samples to check quantitative recovery of starch.

2.2.2 Starch granule morphology

A scanning electron microscope (JOEL model 6400V, Tokyo, Japan) was used to observed kiwifruit starch granules. Kiwifruit starch powders, spread on silver tape and mounted on a brass disk, were coated with gold/palladium (60/40) and observed at 1500 × magnification for all three replicates.

2.2.3 Starch crystallinity

Crystallinity of starch granules was determined using X-ray diffractometry. X-ray diffraction patterns were obtained with copper K_α radiation using a Siemens D-500 diffractometer (Siemens, Madison, WI). Analysis was conducted following the procedure reported by *Song* and *Jane* [7] in which kiwifruit powders were equilibrated to 100% humidity for 24 h prior to analysis and scanned from 4–37 2θ. Degree of crystallinity was calculated based on method of *Hayakawa* et al. [8] using the following equation:

$$\text{Crystallinity (\%)} = A_c/(A_c + A_a) \times 100$$

where A_c = crystalline area on the X-ray diffractogram and A_a = amorphous area on the X-ray diffractogram.

2.2.4 Molar mass and gyration radius of amylopectin

Weight-average molar mass, polydispersity, *z*-average gyration radius and density of amylopectin were determined using high-performance size-exclusion chromatography equipped with multi-angle laser-light scattering and refractive index detectors (HPSEC-MALLS-RI). Starch samples, duplicate measurements of each replicate, were prepared as described by *Yoo* and *Jane* [9]. The HPSEC system consisted of a HP 1050 series isocratic pump (Hewlett Packard, Valley Forge, PA), a multi-angle laser-light scattering detector (Dawn DSP-F, Wyatt Tech. Co., Santa Barbara, CA) and a HP 1047A refractive index detector (Hewlett Packard, Valley Forge, PA). To separate amylopectin from amylose, a Shodex OH pak KB-guard column and KB-806 and KB-804 analytical columns (Showa Denko K.K., JM Science, Grand Island, NY) were used. Operating conditions and data analysis are described by *Yoo* and *Jane* [10], except flow rate used was 0.4 mL/min and sample concentration was 0.8 mg/mL.

2.2.5 Apparent and absolute amylose content

Apparent and absolute amylose content of kiwifruit starch was determined following the procedure of *Lu* et al. [11]. Analysis was based on iodine affinities of defatted whole starch and amylopectin fraction using a potentiometric autotitrator (702 SM Titrino, Brinkmann Instrument, Westbury, NY). Starch samples were defatted using a 90% dimethyl sulfoxide (DMSO) solution, followed by alcohol precipitation. Determination of amylose content was duplicated for each replicate.

2.2.6 Amylopectin branch chain-length distribution

Amylopectin was fractionated by selective precipitation of amylose using *n*-butanol as a complexing agent [12]. Amylopectin (2 mg/mL) was defatted in boiling 90%

DMSO (100°C) for 1 h, followed by stirring for 24 h and then debranched using isoamylase (EC 3.2.1.68 from *Pseudomonas amyloderamosa*) (EN102, Hayashibara Biochemical Laboratories Inc., Okayama, Japan) as described by *Jane* and *Chen* [13]. Branch chain-length distribution of amylopectin was determined using an HPAEC system (Dionex-300 and Dionex-GP50 gradient pump, Sunnyvale, CA) equipped with an amyloglucosidase (EC 3.2.1.3, from *Rhizopus* mold, A-7255, Sigma Chemical Co., St Louis, MO) post-column, on-line reactor and a pulsed amperometric detector (Dionex-ED50, Sunnyvale, CA) (HPAEC-ENZ-PAD) [14]. PA-100 anion exchange analytical column (250 × 4 mm, Dionex, Sunnyvale, CA) and a guard column were used for separating debranched amylopectin samples. Gradient profile of eluents and operating conditions were described previously [15] except that Chromeleon® version 6.50 software was used. HPAEC-ENZ-PAD analysis was duplicated for each replicate.

2.2.7 Thermal properties of starch

Thermal properties of kiwifruit starch were determined using a differential scanning calorimeter (DSC 2920 modulated, TA Instruments, New Castle, DE). Approximately 4 mg of kiwifruit starch was weighed in an aluminum pan, mixed with 12 mg of deionized water and sealed. Sample was allowed to equilibrate for 2 h and scanned at a rate of 10°C/min over a temperature range of 0–120°C. An empty pan was used as reference. Rate of starch retrogradation was determined using the same gelatinized samples, stored at 4°C for 7 d, and analyzed using DSC as described previously [16]. All thermal properties were carried out in triplicate for each replicate.

2.2.8 Pasting properties of starch

Kiwifruit starch pasting properties were analyzed using a Rapid Visco Analyser (RVA-4, Foss North America, Eden Prairie, MN) [17]. A starch suspension (8%, w/w), in duplicate for each replicate, was prepared by weighing kiwifruit starch (2.24 g, dry starch basis, dsb) into a RVA canister and making up the total weight to 28 g with deionized water. The kiwifruit starch suspension was equilibrated at 30°C for 1 min, heated at a rate of 6.0°C/min to 95°C, maintained at 95°C for 5.5 min, then cooled to 50°C at a rate of 6.0°C/min and maintained at 50°C for 5 min. Constant paddle rotating speed (160 rpm) was used throughout the entire analysis except for a speed of 960 rpm for the first 10 s to disperse the sample.

2.2.9 Statistical analysis

All statistical significance tests were calculated using SAS software [18] and applying Tukey difference test [19].

3 Results and Discussion

3.1 Starch structural characteristics

Starch content of kiwifruit (43.4% dry weight) (Tab. 1) was comparable to starch content of other high-moisture-content fruit such as apple [20] and some winter squash [2]. Scanning electron micrographs of kiwifruit starch are shown in Fig. 1. Unlike the findings of *Sugimoto* et al. [5], who reported most kiwifruit starch granules were in the 6–8 μm diameter range, in our study we find a high proportion of starch granules in the 4–5 μm and 7–9 μm range (Fig. 1A). Many kiwifruit starch granules were dome-

Tab. 1. Kiwifruit starch content and structural characteristics. Values after ± represent the standard error of the mean.

Structural characteristic	Kiwifruit starch
Starch content of kiwifruit [% dry weight]	43.4 ± 1.0
Moisture content of kiwifruit [%]	82.9 ± 0.2
Crystallinity [%]	26.8 ± 0.2
Iodine affinity of whole starch	8.57 ± 0.19
Iodine affinity of amylopectin fraction	4.82 ± 0.08
Apparent amylose [%][1]	43.1 ± 1.0
Absolute amylose [%][2]	18.8 ± 0.4
Amylopectin molar mass [g/mol][3,4,5]	$7.36 \times 10^7 \pm 2.6 \times 10^6$
Amylopectin polydispersity (M_w/M_n)[3,4]	1.54 ± 0.04
Amylopectin gyration radius (R_z)[3,4,6]	200.1 ± 3.0
Amylopectin density [g mol^{-1} nm^{-3}][3,4,7]	9.2 ± 0.3

[1] Apparent amylose contents were averaged from two analyses for each of three replicates. Values were calculated from dividing iodine affinity by a factor of 0.199.
[2] Absolute amylose contents were averaged from two analyses for each of three replicates. Values were calculated by subtracting iodine affinity for the amylopectin fraction from the iodine affinity for the whole starch, divided by a factor of 0.199.
[3] Data were obtained from two injections each of all three replicates.
[4] All samples were dissolved in 90% DMSO solution and precipitated with 5 vol. ethanol; freshly prepared starch aqueous solution (100 μL; 0.8 mg/mL) was injected to HPSEC system.
[5] weight-average molar mass.
[6] *z*-average radius of gyration.
[7] Density is equal to M_w/R_z^3.

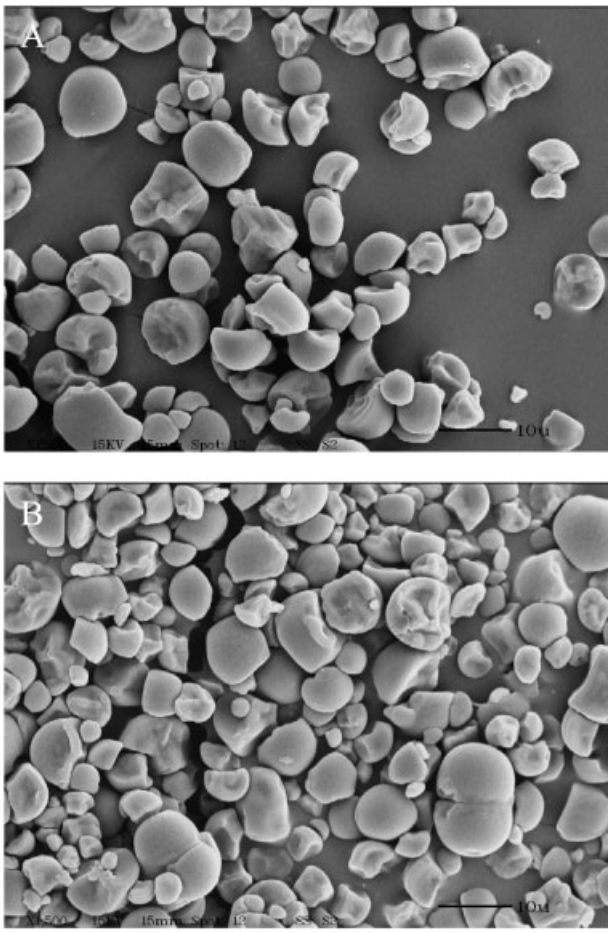

Fig. 1. Scanning electron micrographs of the range of kiwifruit starch granule morphologies (A) and evidence of compound starch granules splitting apart (B). Scale bar = 10 μm.

shaped indicating that kiwifruit starch could consist of compound granules and some granules appeared to be in the process of splitting apart (Fig. 1B).

The kiwifruit X-ray diffraction pattern is shown in Fig. 2. The strong peak at 2θ = 17.2° indicates that kiwifruit starch has a B-type X-ray diffraction pattern which is in agreement with that reported by *Sugimoto* et al. [5], but there is no obvious split peak at 2θ = 22–24°, suggesting that some A-type polymorphs may be present. Kiwifruit starch percent crystallinity was 26.8% (Tab. 1) which is within the range reported for a variety of A- and B-type starches [21].

Iodine affinities and corresponding apparent and absolute amylose contents of kiwifruit starch are shown in Tab. 1. Iodine affinity of the whole starch and its corresponding apparent amylose content were high relative to other non-mutant starches studied [17] but was similar to that

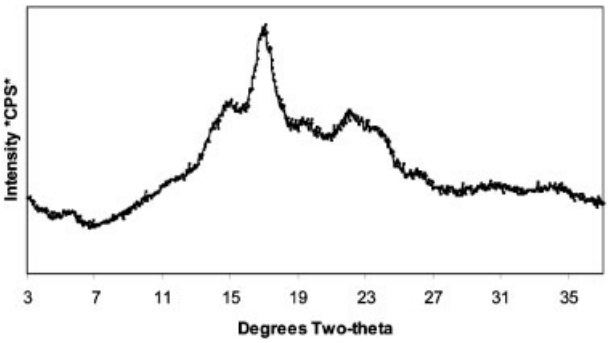

Fig. 2. X-ray diffraction pattern of kiwifruit starch.

reported for apple starch [20]. Amylopectin-fraction iodine affinity was typical for a B-type starch resulting in absolute amylose content of just 18.8%, indicating that kiwifruit amylopectin has considerable amount of chain-lengths greater than DP 40. The absolute amylose content of kiwifruit is comparable to that of potato starch (16.9–19.8%, [17, 22], but slightly lower than that of corn (21.4–22.5% [17, 23]), rice (20.5% [17]) and wheat starch (21.6–25.8% [17, 22–24]).

Kiwifruit amylopectin molar mass, polydispersity, gyration radius and density are shown in Tab. 1. Weight-average molar mass and gyration radius of kiwifruit amylopectin are low relative to all other starches studied with only tapioca starch exhibiting a similar structure [10]. The density of kiwifruit starch was higher than that of all other B-type starches reported except ae waxy maize [10] and squash fruit [25] starch.

Amylopectin branch chain-length distribution of kiwifruit starch is shown in Tab. 2. The long average amylopectin branch chain-length (DP 28.6) for kiwifruit starch is similar to that reported for other B-type starches [17]. Kiwifruit starch has a high proportion of long amylopectin branch-chains (DP $\geq$ 37) (Fig. 3), which is also in agreement with other B-type starches [17, 25]. Average chain-length of kiwifruit amylopectin was longer than that of all A-type

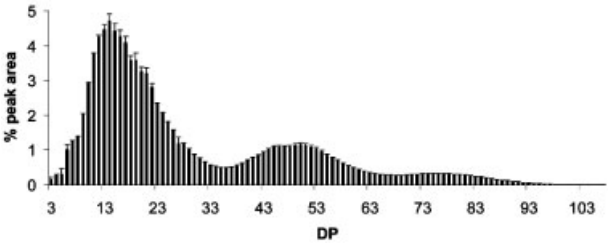

Fig. 3. Relative peak area distribution of kiwifruit amylopectin branch chain-lengths analyzed by using a HPAEC-ENZ-PAD. Error bars represent standard error of the mean for each individual DP from two analyses of three replicates. DP = Degree of polymerization.

Tab. 2. Branch chain-length distributions of kiwifruit starch amylopectins[1].

	Peak DP		Average CL	Percent distribution						Highest detectable DP
	I	II		DP 3–5	DP 6–9	DP 6–12	DP 13–24	DP 25–36	DP $\geq$ 37	
Kiwifruit starch	14	50	28.6	0.7	5.7	20.0	40.1	11.1	28.8	107
SE mean[2]			0.4	0.2	0.2	0.2	1.0	0.2	1.0	

[1] Grouping of degree of polymerization (DP) numbers followed that of *Hanashiro* et al. [29].
[2] SE mean = standard error of the mean.

Tab. 3. Thermal properties of native and retrograded kiwifruit starch.

	Starch gelatinization			Amylose-lipid transition		
	T_o [°C][2]	T_p [°C]	ΔH [J/g]	T_o [°C]	T_p [°C]	ΔH [J/g]
Kiwifruit native starch[1]	68.9 $\pm$ 0.2	73.0 $\pm$ 0.2	18.5 $\pm$ 0.3	91.7 $\pm$ 3.4	100.7 $\pm$ 2.4	6.7 $\pm$ 1.4
Kiwifruit retrograded starch[1]	40.3 $\pm$ 0.5	60.7 $\pm$ 1.1	7.0 $\pm$ 0.5	85.8 $\pm$ 1.6	92.7 $\pm$ 0.3	0.9 $\pm$ 0.2

[1] Starch samples (~4.0 mg, dsb) and deionized water (~12.0 mg) were used for the analysis; T_o, T_p, and ΔH are onset and peak gelatinization temperature, and enthalpy change of gelatinization, respectively.
[2] Values were calculated from three analyses for each of three replicates.

starches (DP 18.8–27.6) [17] and all C-type starches (DP 25.4–26.7) [17] except apple starch (DP 27.9–29.6) [20]. Average amylopectin branch chain-length of kiwifruit starch was comparable with potato (29.4 [17]) but longer than that of corn, rice and wheat starches (all $\leq$ 24 [17]). The average amylopectin branch chain-length and branch-chains with DP $\geq$ 37 is sufficient to explain the large difference between absolute and apparent amylose contents of kiwifruit starch, since a previous study [17] has shown that amylomaize V, amylomaize VII, potato and canna starches have a percentage apparent amylose content of 52.0, 68.0, 36.0 and 43.2, respectively, but a percentage absolute amylose content of 27.3, 40.2, 16.9 and 22.7, respectively. These four starches, listed in same order, have an average amylopectin branch chain-length of DP 28.9, 30.7, 29.4 and 28.9, respectively, and percentage of branch-chains of DP $\geq$ 37 of 26.1, 29.5, 28.9 and 26.8, respectively. Therefore kiwifruit is similar to these other B-type starches that have large differences between absolute and apparent amylose content.

3.2 Starch functional properties

Thermal properties of native and retrograded kiwifruit starch are shown in Tab. 3. Onset gelatinization temperature of kiwifruit starch is higher than that of most non-mutant starches studied [17] and this is also reflected in a relatively higher enthalpy change of starch gelatinization. The onset and peak gelatinization temperatures we observe for kiwifruit starch from 'Hayward' variety fruit are 7–8°C higher than that reported by *Sugimoto* et al. [5] studying the same kiwifruit variety. An amylose-lipid thermal transition peak was not reported by *Sugimoto* et al. [5] for kiwifruit starch. Retrograded kiwifruit starch has not been previously studied. Onset temperature and enthalpy change for melting the retrograded kiwifruit starch was typical compared to other starches studied but peak temperature was abnormally high (47.7°C-57.6°C for all other non-mutant starches) [17]. Thermal transition temperatures decreased after retrogradation for the amylose-lipid complex and this could be due to the double-transition behavior of amylose-lipid complexes from crystalline to an amorphous state that has been observed in cereal starches [26–28].

Pasting properties of kiwifruit starch are shown in Tab. 4 and Fig. 4. Relative to other starches studied at an 8% (w/w) starch concentration, kiwifruit starch has a very high peak viscosity with only lotus root and potato starch reported to be higher [17]. Final viscosity is also higher than that of most starches studied at 8% concentration with only mungbean, green banana and green leaf canna starches exhibiting higher final viscosities [17]. Setback was also relatively high indicating sufficient amylose and amylopectin mobility for coprecipitation to occur. One unusual characteristic of kiwifruit starch is that its pasting temperature is marginally higher than the onset gelatini-

Tab. 4. Pasting properties of kiwifruit starch measured by Rapid Visco Analyser.

	Peak viscosity[2]	Trough[2]	Break-down[2]	Final viscosity[2]	Setback[2]	Pasting temperature [°C]
Kiwifruit starch[1]	250 ± 14	144 ± 5	106 ± 9	238 ± 8	94 ± 6	69.7 ± 0.1

[1] 8% (w/w) kiwifruit starch suspension measured in duplicate for all three replicates
[2] Viscosity measured in Rapid Visco Analyser units (RVU), 1 RVU = 12 mPas.

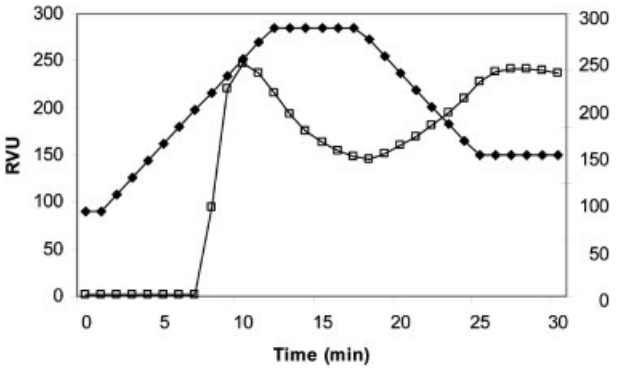

Fig. 4. Rapid Visco Analyser pasting profile of kiwifruit starch (8.0% dsb, w/w).

zation temperature. Th pasting temperature of 70°C for kiwifruit starch was higher than that of potato (63.5°C) but considerably lower than that of cereal starch from rice (79.9°C), corn (82.0°C) and wheat (88.6°C) [17].

4 Conclusions

Kiwifruit starch is B-type with granules 4–9 μm in diameter and absolute amylose content of 18.8%. Kiwifruit amylopectin molecular weight is small relative to other starches. Kiwifruit starch had very high peak, final and setback viscosities relative to other starches and pasting temperture was marginally higher than onset gelatinization temperature. Kiwifruit starch could be suitable for sauces, gravies and soups where its high paste viscosity and pasting temperature marginally higher than onset gelatinization temperature would allow complete paste dispersion to occur at low temperatures and provide a smooth texture to foods due to absence of ungelatinized starch granules. Kiwifruit starch pasting temperature of 70°C is advantageous over many cereal starches such as corn and rice that paste at 10–12°C higher and wheat, barley, rye and oat starch that paste 18–25°C higher. Therefore food processors can conserve heat energy in obtaining similar paste viscosity and consumers can benefit from less grainy texture in foods where processing does not exceed 80°C. Although not as suitable as the very small starch granules of amaranth starch, the smaller granule size distribution to corn starch and absence of granules greater than 10 μm in diameter suggest that kiwifruit starch could possibly have applications as a fat mimetic.

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